that TatA forms larger complexes upon substrate binding in the presence of a membrane potential.

275-Pos
Solvation and Binding of the Membrane Enzyme Pagp By Detergents and Lipids
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The solvation of membrane proteins by detergents is a necessary step for structure determination by NMR and X-ray crystallography, and yet this process remains poorly understood. The severe under-representation of membrane proteins amongst proteins of known structure is a direct consequence of the difficulties associated with the solubilization of the large hydrophobic faces presented by this important class of proteins. The prominence of these membrane proteins as important drug targets provides a strong impetus for the rational design of new detergents or cofactors to assist the solubilization of hydrophobic faces while maintaining structural integrity. To this end, we begin by investigating the process of detergent self-aggregation, utilizing molecular dynamics simulations to characterize the atomic interactions that underlie the dynamic aggregation of detergents in aqueous solution. We determine the critical micelle concentration and the equilibrium aggregation number based on generalized ensemble methods and, separately, thermodynamic cycles involving non-physical order parameters that are more computationally efficient. Next, we apply similar methods to study the aggregation of detergents around the bacterial outer membrane enzyme and virulence factor PagP. These studies shed light on the process of protein self aggregation in solution and the atomistic mechanisms by which detergents may prevent protein precipitation at high concentrations. Finally, we draw functional conclusions for PagP, an acyltransferase that binds and catalyses its own lipid solvent.

276-Pos
Protein Secondary Structure Prediction Using Knowledge-Based Potentials and An Ensemble of Classifiers
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A novel method is proposed for predicting protein secondary structure using knowledge-based potentials and Neural Networks. Potential energies for amino acid sequences in proteins are calculated using protein structures in the CATH database. The data consists of energy information calculated for a reduced set of three secondary structures: alpha-helix, beta-strand and coil. An Extreme Learning Machine (ELM) classifier, based on Neural Network, is used to model and predict protein secondary structure from this data.
Other classification techniques such as Support Vector Machines (SVM) and Naïve Bayes (NB) are also used and show comparable performance. Preliminary results show that an ensemble of various techniques can collectively improve prediction results.

277-Pos
Determination of the Mechanism of Selectivity and Ammonia Conduction By AmtB Using MD Simulations
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The transport of ammonia, fundamental to the nitrogen metabolism in all domains of life, is carried out by the Rh/Amt/MEP membrane protein superfamily. The first structure of this family (AmtB from E. coli) shows a pathway for ammonia that includes two vestibules connected by a long and narrow hydrophobic lumen. The accepted mechanism for AmtB is to recruit NH4+ and conduct neutral NH3 by deprotonation of NH4+ at the end of the periplasmic vestibule. We conducted several MD simulations (total of more than 0.3μs) using a model of trimeric AmtB embedded into POPE lipid bilayer to determine the mechanism of ligands conduction in the ammonia channels.
To determine the AmtB's selectivity, we added 100 ligand molecules to our solvated protein-lipid system and conducted unconstrained MD simulations for each ligand. The probability distribution for each ligand along the normal of the lipid plane shows that the periplasmic vestibule prefers NH4+ over NH3 and CO2. Our long MD simulations reveal that two stacking phenyl rings of F107 and F215 (located at the bottom of the periplasmic vestibule) simultaneously flip open and close with a frequency of ~108 flip-open events per second. The frequency of flip open/close events is independent of the presence of NH4+ at the vestibule. This indicates that the rate of this channel is controlled not only by the concentration gradient of ammonia but also by the frequency of phenyl rings open/close events.
Our simulations show that D160 along with the aromatic rings are essential for recruitment of NH4+ at the phenyl rings gate. In each ammonia conduction cycle, NH4+ stays behind the gate long enough for the gate to flip open and let ammonium enters the lumen.

278-Pos
Electrophysiology of Viral Envelope Protein Ion Channels in Lipid Membranes Across Apertures in Polystyrene and Silicon
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Viral ion channels, such as the coronavirus envelope proteins (E protein), belong to a family of channels that have attracted a considerable amount of interest during recent years. However, not many studies on the electrophysiology have been performed; mainly due to the fact that these channels are membrane proteins that do not easily express in the outer membrane of bacteria. Moreover, the channel currents are small (on the order of 10-300 pS) when compared with bacterial outer membrane channels. In our studies, we reconstituted the full-length channel-forming E protein from murine hepatitis coronavirus (MHV-A59) into 3:1:1 POPE:POPS:POPC lipid bilayers that were suspended either across 150 μm diameter apertures in polystyrene cups or across a 50 μm diameter aperture in silicon. Lipid bilayers were formed using the painting method on all substrates, resulting in reproducible Gigaseal formation. The aperture in silicon was prepared using photolithography and dry reactive ion etching, resulting in excellent reproducibility of the pore geometry. The surface was coated hydrophobically to allow lipid bilayer attachment.
Bilayers created in the presence of E-protein in solution showed reproducible ion channel activity, independent of the substrate used. We were able to identify the signature conductance steps of E ion channels. Similar to what has been shown previously using the OmpF ion channel of E. coli, the ion channel activity on the silicon substrate was identical to that measured using the polystyrene cup, indicating the feasibility of the silicon substrate for the investigation of ion channels with conductances in the range of tens of picosiemens. Using silicon apertures for ion channel reconstitution experiments in array geometry provides an opportunity to increase measurement throughput.

279-Pos
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280-Pos
Single Amino Acid Substitutions Change the Sodium/Iodide Symporter (NIS) Selectivity and Stoichiometry
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The Na^+/I^- symporter (NIS) is a key plasma membrane protein that mediates active I^- transport in the thyroid and such other tissues as salivary glands, stomach, and lactating breast. NIS-mediated I^- uptake is the first step in thyroid hormone biosynthesis. NIS mediates the inward simultaneous movement of Na^+ and I^- with a 2:1 stoichiometry, thus resulting in a net transfer of positive charge into the cell (i.e., electrogenic transport). We recently reported that NIS translocates different anion substrates with different stoichiometries, as Na^+/perchlorate (or perrhenate) transport is electroneutral. Valuable mechanistic information on NIS has been obtained by the characterization of NIS mutants that cause congenital I^- transport defect in patients. Here we provide a detailed study of the G93R NIS mutant. As we substituted neutral amino acids at this position, we observed that the longer the side chain of the substituted residue, the lower the protein's activity. G93T and G93N NIS exhibited significantly higher K_m values for I^- than WT NIS, the first time that such a change has been observed in any NIS mutants. Strikingly, we show by kinetic analysis that G93T-mediated Na^+/perrhenate symport is electrogenic with a 2:1 stoichiometry, a discovery confirmed by the detection of currents elicited by perrhenate (or perchlorate) in G93T NIS-expressing *X. laevis* oocytes in electrophysiological experiments. These observations demonstrate that a single amino acid substitution at position 93 converts NIS-mediated Na^+/perchlorate (or perrhenate) transport stoichiometry from electroneutral to electrogenic. Based on the 3-D structure of the bacterial Na^+/galactose transporter, we built a 3-D homology model of NIS and we propose a mechanism in which changes from an outwardly open to an inwardly open conformation during the transport cycle use G93 as a pivot.